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Differential Regulation of the Myosin Heavy Chain Genes α and β in Rat Atria and Ventricles: Role of Antisense RNA

Sara Danzi,[1,2] Steven Klein,[1] and Irwin Klein[1–3]

Background: The myosin heavy chain (MHC) genes are regulated by triiodothyronine (T_3) in a reciprocal and chamber-specific manner. To further our understanding of the potential mechanisms involved, we determined the T_3 responsiveness of the MHC genes, α and β, and the β-MHC antisense (AS) gene in the rat ventricles and atria.

Methods: Hypothyroid rats were administered a single physiologic (1 μg) or pharmacologic (20 μg) dose of T_3, and sequential measurements of β-MHC hn- and AS RNA and α-MHC heterogeneous nuclear RNA from rat ventricular and atrial myocardium were performed with reverse transcription PCR.

Results: We have demonstrated that T_3 treatment increases the myocyte content of an AS β-MHC RNA in atria and ventricles that includes sequences complementary to both the first 5′ and last 3′ introns of the β-MHC sense transcript. In the hypothyroid rat ventricle, β-MHC sense RNA expression is maximal, while in the euthyroid rat ventricle, β-MHC AS RNA is maximal. β-MHC AS expression increased by $52 \pm 9.8\%$ at the peak, 24 hours after injection of a physiologic dose of T_3 (1 μg/animal), while β-MHC sense RNA decreased by $41 \pm 2.2\%$ at 36 hours, the nadir. In hypothyroid atria, β-MHC AS RNA was induced by threefold within 6 hours of administration of 1 μg T_3, demonstrating that in the atria, β-MHC AS expression is regulated by T_3, while α-MHC expression is not.

Conclusions: In the hypothyroid rat heart ventricle, β-MHC AS RNA expression increases in response to T_3 similar to that of α-MHC. Simultaneous measures of β-MHC sense RNA are decreased, suggesting a possible mechanism for AS to regulate sense expression. In atria, while α-MHC is not influenced by thyroid state, β-MHC sense and AS RNA were simultaneously and inversely altered in response to T_3. This confirms a close positive relationship between T_3 and β-MHC AS RNA in both the atria and ventricles, while demonstrating for the first time that α- and β-MHC expression is not coupled in the atria.

Introduction

Thyroid hormone exerts multiple effects on the heart and cardiovascular system (1). Triiodothyronine (T_3) is taken up by the cardiac myocyte and enters the nucleus, where it binds to T_3 nuclear receptor proteins (TRs), which in turn bind to thyroid hormone response elements (TREs) in the promoter regions of positively regulated genes (2–5). In the presence of T_3, transcription is induced; in the absence of T_3, transcription is repressed (2,3). The cellular mechanisms of T_3 action have been well worked out for the induction of the cardiac-specific α-myosin heavy chain (α-MHC) gene; however, the pathways for the negatively regulated β-MHC gene are not well understood. The β-MHC promoter does not contain a consensus TRE, although a putative half site has been identified (6–8). A variety of lines of evidence suggest that the T_3-mediated regulation of β-MHC transcription occurs posttranscriptionally (9–11).

The MHC genes encode the proteins that form the thick filament of the contractile apparatus in the cardiac myocyte. In the euthyroid rat heart, α-MHC predominates, while in the human heart, β-MHC predominates in all thyroid states. Expression of the MHC genes is altered in response to several physiologic changes in addition to thyroid status (Table 1). The mechanism responsible for the apparent reciprocal expression of the two MHC isoforms is not fully understood. Others and we have identified the presence of β-MHC antisense (AS) RNA in rat cardiac myocytes (10,14–18). The β-MHC AS transcript has been identified as a full-length heterogeneous nuclear RNA (hnRNA) molecule that includes

[1]Feinstein Institute for Medical Research and the Department of Medicine, North Shore University Hospital, Manhasset, New York.
Departments of [2]Medicine and [3]Cell Biology, NYU School of Medicine, Manhasset, New York.

Table 1. MHC Isoform Switching in the Cardiac Myocyte

Condition	*Physiologic response*
Rat	
T_3 treatment ↑ α-MHC ↓ β-MHC	↑ speed of contraction, ↑ cardiac growth
Aging ↓ α-MHC ↑ β-MHC	↓ speed of contraction, cardiac atrophy
Pathologic hypertrophy ↓ α-MHC ↑ β-MHC	↑ cardiac growth, heart failure
Human	
T_3 treatment No change[a]	Cardiac growth secondary to increased cardiac work
Heart failure ↓ α-MHC β-MHC same	Impaired systolic and diastolic function

[a]In profound hypothyroidism α-MHC does respond to T_4 treatment (12,13).

MHC, myosin heavy chain; T_3, triiodothyronine; T_4, thyroxine.

the corresponding intronic and exonic regions, and is referred to as the AS RNA (10,14,15,18). In the present study, we report for the first time that the content of the β-MHC AS RNA in the cardiac myocyte varies in response to and directly with serum T_3. The expression of the β-MHC sense RNA and the β-MHC AS RNA transcripts varies inversely in response to thyroid hormone and provides a potential mechanism to further our understanding of MHC regulation in the mammalian heart.

Materials and Methods

Animal protocols

Adult male Sprague-Dawley rats (6–8 weeks old) were obtained from Taconic Farms (Germantown, NY). Animals were rendered hypothyroid by surgical thyroidectomy (Tx). Seven days after surgery, hypothyroidism was confirmed by analysis of serum total thyroxine (T_4) and total T_3 levels by RIA (Diasorin, Stillwater, MN). Matched euthyroid (Eu) animals were used for controls. T_3 treatment was accomplished by an intramuscular (IM) injection of 1 μg T_3 (5 μg/kg body weight) (ICN Biomedicals, Aurora, OH) in 0.2 mL PBS, and animals were killed at 6, 12, 24, 36, 48, and 72 hours after injection. Additional rats were given a second 1 μg T_3 injection 24 hours later and killed at 48 hours. In a second study, rats were given an IM injection of 20 μg T_3 (100 μg/kg body weight) in 0.2 mL PBS and killed after 6, 12, 24, or 36 hours. Hearts were quickly excised; right and left atria were identified and removed, and then weighed; left ventricles including septum were dissected, weighed, and all myocardial tissue was rapidly frozen in liquid nitrogen and stored at −80°C until extracted for RNA. Three or four animals were used for each experimental time point. Blood was collected for serum T_3 analysis.

The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication No. 85–23, revised 1985).

Total RNA isolation

Total RNA was extracted from frozen rat atrial and left ventricular (LV) tissue as we have previously described (19). Pretreatment with DNase I (Qiagen, Valencia, CA) ensured that no amplification of the cardiac genomic DNA occurred. Fifty micrograms of total RNA was treated with DNase I and RNeasy mini protocol for RNA Cleanup (Qiagen). RNA concentration was determined spectrophotometrically, and integrity of the RNA was confirmed using the Agilent 2100 bioanalyzer.

Reverse transcription PCR–based assay to determine sense and AS RNA expression

In the current report, transcription of the MHC genes is measured by quantitation of the primary transcript as previously described (20). To assess this, total RNA from the hearts of experimental animals was used in a reverse transcription (RT) reaction with a reverse primer amplifying the sense hnRNA. RT was performed with 2 μg of total LV RNA and reverse primers that annealed to sequences at the 5′ end (primer set 1) or at the 3′ end (primer set 2) of the β-MHC gene (GenBank Acc. No. X16291) in a total volume of 50 μL as previously described (Table 2) (20). These primers amplified β-MHC sense RNA. Specific primers for α-MHC hnRNA have been previously described (20). RT was accomplished with Moloney Murine Leukemia Virus Reverse Transcriptase (Promega, Madison, WI). Because of the potential for Taq polymerase to have reverse transcriptase

Table 2. PCR Primer Sequences Targeting β-MHC Sense and AS Transcripts

	Sequence	*Location*	*Fragment size*
	Primer set 1		
β-MHCF (exon)	5′-TGAGCATTCTCCTGCTGTTTC-3′	5′ end of β-MHC sense strand	312 bp
β-MHCR (intron)	5′-ACACACGCGCACACACTAGCA-3′		
	Primer set 2		
β-MHCF(b) (intron)	5′-ATCCCTCAAGGTCACACAAGG-3′	3′ end of β-MHC sense strand	193 bp
β-MHCR(b) (exon)	5′-CTCCAGGTCTCAGGGCTTCAC-3′		

MHC, myosin heavy chain; AS, antisense; F, forward; R, reverse.

activity, we routinely treat the completed RT reactions with 1 μL RNase A (Ambion, Austin, TX) at 37°C for 20 minutes prior to PCR (9,21). Negative controls that contained reverse transcriptase without primers and controls without reverse transcriptase were also included, and these produced negligible or no PCR products (18,21,22).

To quantitate β-MHC AS RNA in rat hearts, we performed RT on 2 μg total RNA as described above, but used the forward primer from each primer set (Table 2) to synthesize the cDNA copy of the AS hnRNA. Primer sets 1 and 2 annealed to sequences at the 3′ and 5′ ends of the β-MHC AS transcript, respectively. Specifically, primer set 2 annealed to a region that corresponds to the last intron and 3′ UTR of the β-MHC sense transcript.

Following RT and RNase digestion, PCR amplification of a 312 or 193 bp fragment of β-MHC was accomplished with β-MHC primer sets 1 and 2, respectively. The primer sequences for PCR amplification of the 335 bp fragment of α-MHC were previously published (20). Using an aliquot of the RT reaction, PCR was performed using Amplitaq Gold enzyme (Perkin Elmer, Foster City, CA) as previously described (20). PCR products were run on a 2% agarose gel with ethidium bromide and quantitated by densitometry using BioRad Quantity 4.2.2. Software. All RT reactions were done in duplicate.

All β-MHC sense and AS PCR products were sequenced and confirmed using an ABI prism 3100 Genetic Analyzer (Applied Biosystems/Hitachi, Foster City, CA).

Preparation of standard PCR products

For quantitation of β-MHC sense RNA, 2 μg RNA from each of three Tx control hearts was used for RT reactions with each of the β-MHC reverse primers, and PCR was accomplished using both reverse and forward β-MHC primers for each primer set as described above. Aliquots of the RT reactions were used for PCR, and all reactions were pooled for use as a standard. The same procedure was used for quantitation of β-MHC AS RNA using RNA from three Eu control hearts. Each time experimental PCR products were quantitated by densitometry, we included PCR product corresponding to 4, 8, 20, 40, and 60 ng of input RNA of the appropriate standard. A standard curve was generated, and data were expressed as density units per ng of input RNA. The data from this curve were used to quantitate the gene expression of individual samples and are expressed as a percent of the control.

Statistical analysis

All data are expressed as the mean $\pm$ SE. Statistical differences between values were evaluated by Student's *t*-test with significant probability at $p < 0.05$.

Results

Cardiac mass and thyroid status

Serum T_3 and T_4 were measured 8 days after thyroidectomy (Tx) and before treatment to ensure chemical hypothyroidism. Serum T_3 levels and LV weights were measured in hypothyroid and euthyroid control rats and at the times indicated after T_3 treatment in each experiment (Table 3) (20). Serum T_3 levels were 29 ± 2.9 ng/dL and 122 ± 13.6 ng/dL in hypothyroid and euthyroid animals, respectively. Heart weights (LV) confirmed the hypothyroid state in these animals and were 386 ± 18.0 mg and 480 ± 19.4 mg in hypothyroid and euthyroid animals, respectively ($p < 0.01$). Serum T_3 levels were 270 ± 24.1 ng/dL at 6 hours after treatment with 1 μg T_3. Serum T_3 levels returned toward baseline by 24 hours after treatment with 1 μg T_3 (65 ± 7.0 ng/dL). After T_3 treatment to hypothyroid rats, there was no change in heart weight or heart weight/body weight ratios.

Table 3. Serum T_3 and Cardiac Mass in Control Rats and after Administration of T_3

	Serum T_3 (ng/dL)	*LV weight (mg)*
Hypothyroid	29 ± 2.9	386 ± 18.0
Euthyroid	122 ± 13.6[a]	480 ± 19.4[b]
1 μg T_3		
6 hours	270 ± 24.1[a]	ND
12 hours	150 ± 21.3[a]	ND
24 hours	65 ± 7.0[a]	397 ± 23.0
48 hours (2nd injection at 24 hours)	57 ± 1.7[a]	378 ± 10.7

[a] $p < 0.01$ versus hypothyroid.
[b] $p < 0.01$ versus all other groups.
T_3, triiodothyronine; ND, not determined; LV, left ventricular.

Measurements of α- and β-MHC hnRNA in hypothyroid animals in response to treatment with T_3

Measurements of α- and β-MHC hnRNA were accomplished with primers that annealed to sequences within the 5′ region of each gene. The primer sets for each gene targeted the first intron (reverse primer) and exon (forward primer) within each of the hnRNA molecules. In hypothyroid rats, the expression of α-MHC hnRNA was not detectable. Rats were administered 1 μg T_3 as described. Full α-MHC transcription was observed at 6 hours after treatment, confirming that 5 μg T_3/kg body weight is a sufficient receptor saturating dose (20). Subsequently, α-MHC transcription declined in parallel with serum T_3 levels, which had returned to hypothyroid levels by 24 hours.

In contrast, the expression of β-MHC hnRNA is maximal in hypothyroid animals and is negatively regulated by T_3. Six hours after administration of T_3, β-MHC hnRNA expression declined to 86 ± 1.7% of hypothyroid, and expression continued to decline reaching a nadir at 36 hours, at 59 ± 2% of hypothyroid after a single dose of 1 μg T_3 (Fig. 1) (9,20).

Measurements of β-MHC AS RNA in response to treatment with T_3

Full-length β-MHC AS RNA has been reported in the mammalian myocardium (10,14,15). Measurements of β-MHC AS RNA were performed using the hnRNA primers that target the 3′ end of the AS molecule (Table 2). To study the potential role of β-MHC AS RNA as a mechanism for the repression of β-MHC hn (sense) RNA expression in response to T_3, both β-MHC sense and AS RNA were simultaneously measured in hypothyroid and euthyroid rat hearts. As previously reported, expression of β-MHC sense RNA was maximal in hypothyroid rat hearts, while β-MHC AS RNA

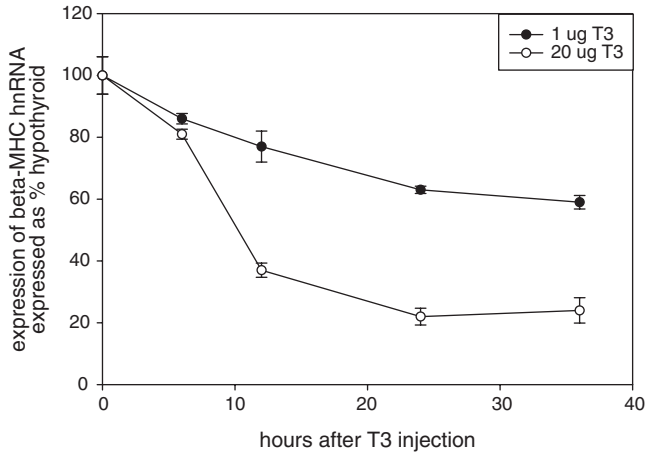

FIG. 1. LV expression of β-myosin heavy chain heterogeneous nuclear RNA (β-MHC hnRNA) (sense) after a single injection of 1 or 20 μg triiodothyronine (T_3) to hypothyroid animals using primer set 1. Values are expressed as percent of the maximal expression in hypothyroid hearts. LV, left ventricular.

was maximal in euthyroid rat hearts. The expression of β-MHC sense RNA in the euthyroid rat heart was measured at $58 \pm 1.3\%$ ($p < 0.01$) when compared to hypothyroid levels. Conversely, the expression of β-MHC AS RNA in the hypothyroid heart was $38 \pm 10.3\%$ of euthyroid AS levels (Fig. 2).

The time course of the simultaneous changes in both β-MHC sense and AS RNA in response to a single 1 μg dose of T_3 is shown in Figure 3. β-MHC AS RNA content increased in response to T_3 and peaked at 24 hours at $102 \pm 9.8\%$ of euthyroid levels. By 48 hours after T_3 treatment, at a time when serum T_3 levels had returned to hypothyroid levels (24 ± 3.2 ng/dL), β-MHC AS levels declined to baseline ($47 \pm 2.9\%$ of euthyroid AS levels). It is interesting to note that β-MHC sense RNA reached the nadir ($59 \pm 2.2\%$ of hypothyroid levels at 36 hours) just after AS RNA levels peaked. As serum T_3 levels fell over the next 24–36 hours, β-MHC sense and AS RNA levels continued to rise and fall, respectively, to pretreatment levels. The data demonstrate for the first time that measurements of β-MHC sense and AS RNA expression are inversely related in response to a single dose of T_3 over 72 hours.

To further demonstrate the role of T_3 in the regulation of β-MHC AS RNA, rats were given a second injection of T_3 24 hours after the first injection and at a time when serum T_3 levels were declining. At 24 hours after the second injection (48 hours total treatment time), β-MHC sense levels decreased significantly from $79 \pm 1.0\%$ to $45 \pm 5.7\%$ (Fig. 2). At the same time, β-MHC AS RNA levels were maintained at $80 \pm 10.5\%$ of euthyroid levels (vs. $47 \pm 2.9\%$ without a second injection) ($p < 0.05$). These data demonstrate that β-MHC sense and AS RNA levels vary in a reciprocal manner in response to changing serum T_3 levels. Simultaneous measurements of β-MHC sense RNA in response to T_3 demonstrated no difference between low (1 μg) and high (20 μg) doses at 6 hours, but by 12, 24, and 36 hours, the high dose of T_3 led to greater suppression ($76 \pm 4.1\%$ vs. $41 \pm 2.2\%$).

In response to 20 μg T_3, β-MHC AS RNA levels were higher at 6 hours after administration of T_3 ($131 \pm 6.6\%$ euthyroid levels) than after the lower dose ($44 \pm 8.5\%$ of euthyroid), and remained high at 36 hours in contrast to AS levels 36 hours after administration of 1 μg T_3 ($77 \pm 2.5\%$ vs. $30 \pm 8.9\%$ of euthyroid levels, respectively) (Fig. 4).

β-MHC AS RNA levels—targeting the 5′ and 3′ ends

In this study and previously, we confirmed that the β-MHC AS RNA is a full-length primary transcript by using primers that targeted the 3′ end of the AS transcript (5′ end of the sense transcript) (15). To better understand the mechanisms involved in AS regulation of the sense transcript, we designed primers to target the 5′ end of the β-MHC AS RNA molecule (primer set 2 in "Materials and Methods" section and Table 2) and compared expression levels targeting the 5′ and 3′ ends of the AS molecule. In the euthyroid myocardium, the signal for β-MHC AS expression was stronger when targeting the 5′ end of the molecule compared to the 3′ end. In hypothyroid animals, both primer sets indicated low levels of β-MHC AS RNA expression. When we target the 5′ end of both sense and AS, we see the same reciprocal relationship (Fig. 5).

β-MHC AS RNA is expressed in rat atrial tissue

Rat atrial myocytes express predominantly α-MHC protein and a smaller amount of β-MHC similar to that of the euthyroid adult ventricle. However, the former is unaffected

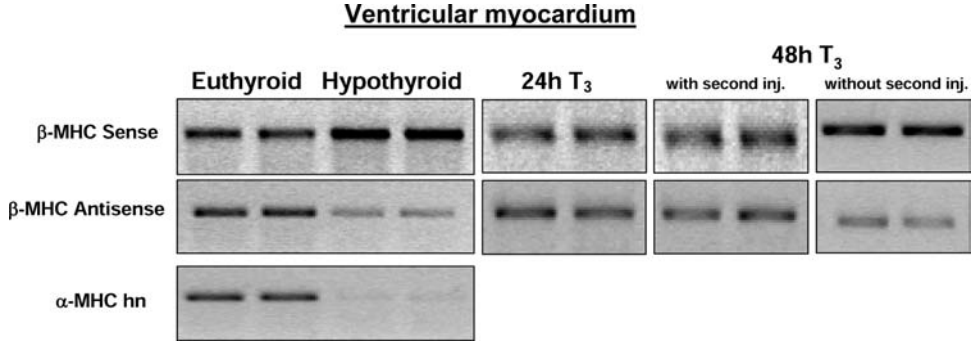

FIG. 2. Representative gels for α- and β-myosin heavy chain (MHC) sense and antisense (AS) RNA from ventricular and atrial cardiac tissue in euthyroid and hypothyroid rat hearts. The 24- and 48-hour bands represent the amount of β-MHC sense and AS RNA in hypothyroid animals after injection of 1 μg triiodothyronine (T_3). Animals sacrificed at 48 hours received a second injection of 1 μg triiodothyronine (T_3) 24 hours after the first injection as described in "Materials and Methods" section.

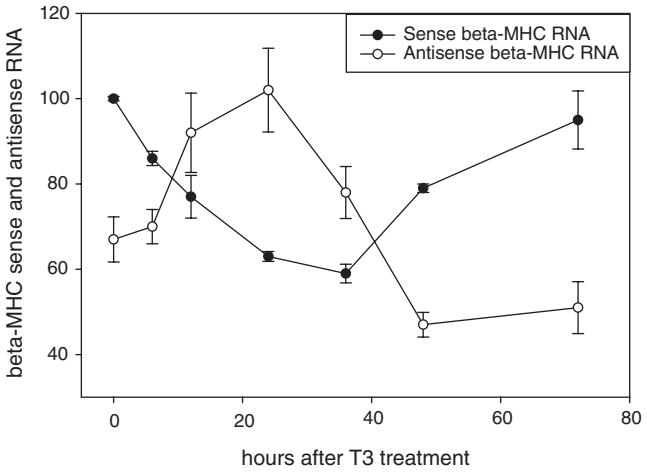

FIG. 3. β-Myosin heavy chain (MHC) sense and antisense (AS) expression after administration of a single 1 μg dose of triiodothyronine (T_3) to hypothyroid rats. Expression of β-MHC sense RNA is given as the percent of hypothyroid levels (where expression is maximal), and expression of β-MHC AS RNA is given as the percent of euthyroid levels (where expression is maximal). Reverse transcription (RT) PCR was accomplished using primer set 1 for both sense and AS RNA. Expression was measured by RT PCR as described at the various time points after a single injection of 1 μg T_3.

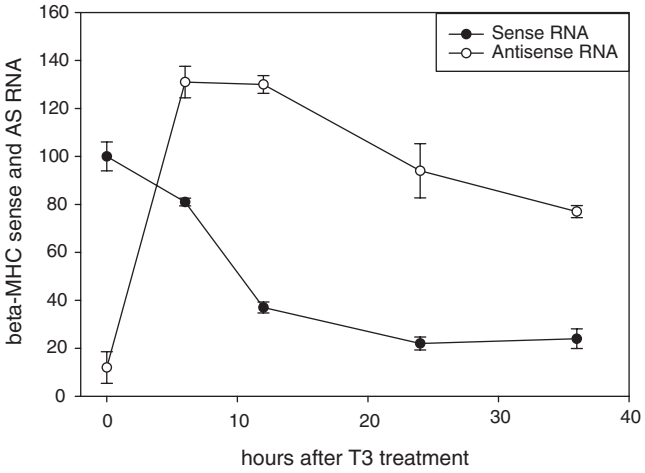

FIG. 5. β-Myosin heavy chain (MHC) sense and antisense (AS) expression after administration of a single 20 μg dose of triiodothyronine (T_3) to hypothyroid rats. Expression of β-MHC sense RNA is given as the percent of hypothyroid levels, and expression of β-MHC AS RNA is given as the percent of euthyroid levels. Reverse transcription PCR was accomplished as described using primer sets that target the 5′ end of each molecule (primer sets 1 and 2 for sense and AS RNA, respectively). Expression was measured at the various time points after a single injection of 20 μg T_3 to hypothyroid rats.

by changes in thyroid status (23–25). Measurements of β-MHC sense and AS RNA in euthyroid and hypothyroid atrial myocardium demonstrate that expression is influenced by thyroid status (Fig. 6). In euthyroid animals, atrial β-MHC sense RNA was approximately 59% of hypothyroid levels ($p < 0.01$) and hypothyroid atrial β-MHC AS RNA was 27% of euthyroid levels ($p < 0.0001$). In hypothyroid atria, β-MHC AS RNA was induced by threefold within 6 hours of administration of 1 μg T_3 (Table 4). These data indicate that in the atria, similar to the ventricle, β-MHC AS expression is regulated by T_3 while α-MHC expression is not. Thus, for the first time, we demonstrated that the expression of α- and β-MHC is not reciprocal and is not coupled to T_3 in the atrium.

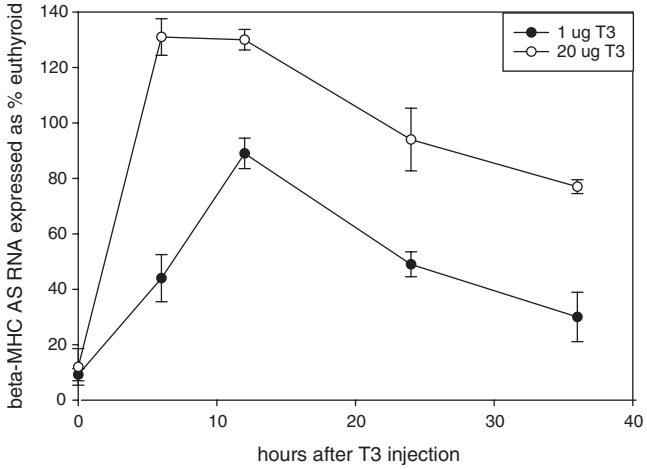

FIG. 4. Expression of β-myosin heavy chain antisense (β-MHC AS) RNA at 6, 12, 24, and 36 hours after a single injection of 1 or 20 μg triiodothyronine (T_3) to hypothyroid animals using primer set 2. Expression is given as percent euthyroid AS levels.

Discussion

In the adult rodent myocardium, α-MHC predominates, while in the human ventricle, β-MHC is the major isoform expressed; although the former is strictly regulated by T_3 in the rodent ventricular myocardium, in the human heart the MHC genes are minimally responsive to thyroid hormone (12,26,27). Since a role for α- and β-MHC isoform switching in regulating cardiac contractility has been proposed, the mechanisms responsible for these species-specific differences are potentially important (23,28,29). The 5′ regulatory regions of both the α- and β-MHC genes are highly homologous in rodents and human, yet the specific sequences that mediate the different patterns of MHC gene expression in these species have not been identified (6,30,31). The expression of the α-MHC gene in rodents has been well studied, and classical TREs that bind TRs have been identified in the promoter region of this gene. The regulatory elements for negatively regulated thyroid hormone responsive genes are not well defined (32). Putative TRE half sites have been identified in the β-MHC promoter region, but the functional significance for these sequences has not been confirmed (7,11,33,34).

MHC isoform gene switching in the heart can occur in response to thyroid hormone, hemodynamic load, or a number of other pathological stimuli, including congestive heart failure (Table 1). It occurs readily in the rodent myocardium and, to a significantly lesser degree, in the human myocardium. Previous work in our laboratory has demonstrated that

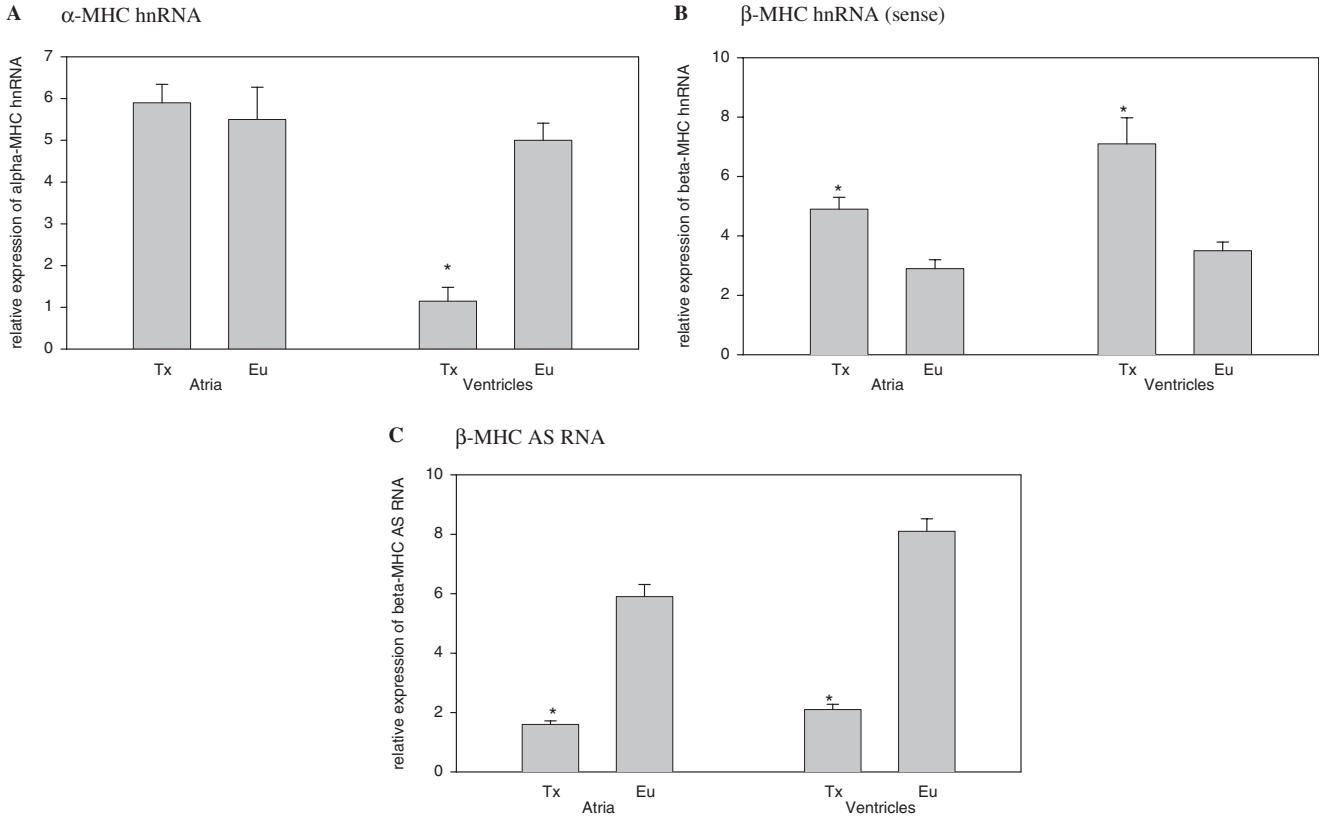

FIG. 6. Expression of (**A**) α-myosin heavy chain heterogeneous nuclear RNA (α-MHC hnRNA), (**B**) *β*-MHC sense (hnRNA), and (**C**) *β*-MHC antisense (AS) RNA in the atria and ventricles of hypothyroid (Tx) and euthyroid (Eu) rats. *$p < 0.01$ versus Eu.

the T_3-mediated regulation of α- and *β*-MHC in the rodent heart occurs with different kinetics (20). The T_3-mediated induction of α-MHC occurs rapidly, while the repression of *β*-MHC in response to T_3 occurs more slowly. *In vivo* studies with actinomycin D to inhibit transcription in rats demonstrated that *β*-MHC expression declined by almost 40% at 2 hours, while in response to T_3, expression had only declined by 14% at 6 hours (9). These observations indicate that the T_3-mediated repression of *β*-MHC occurs by some other mechanism that is distinct from the direct inhibition of transcription and suggests that *β*-MHC is regulated by posttranscriptional mechanisms (9,11).

Others and we have previously identified the presence of an AS transcript for the *β*-MHC gene in the rat that is altered in different thyroid states and in response to diabetes and abdominal aortic constriction, a model of pressure overload (10,15,18). To better understand the mechanism for this regulation, we tested the temporal and dose–response relationship for the T_3-responsiveness of *β*-MHC AS RNA expression. In support of the observation that *β*-MHC AS RNA was high when *β*-MHC sense RNA (hnRNA) was low as occurs in the euthyroid heart, the reverse was true for the expression of *β*-MHC sense and AS RNA in the hypothyroid heart (10). We have demonstrated for the first time that acute T_3 treatment positively regulates the expression of the AS transcript, simultaneously with α-MHC transcription.

These current data suggest that the *β*-MHC AS gene is positively regulated by T_3 and the level of expression is dependent on ambient levels of T_3. The maximal transcriptional response of the *β*-MHC AS gene in response to T_3 occurs with both physiologic and pharmacologic doses of T_3 (15,20). We propose that the transcription of the *β*-MHC gene is constitutive and that the expression of the *β*-MHC sense transcript, which leads to mature *β*-MHC mRNA, is posttranscriptionally regulated (20). We propose that hybridization of sense and AS transcripts to suppress expression of *β*-MHC requires sufficient levels of serum T_3 to maintain AS transcription. Ultimately, *β*-MHC AS RNA transcription regulates the

Table 4. Expression of α-MHC hnRNA and *β*-MHC AS RNA in Euthyroid and Hypothyroid Rat Atrial Tissue and after T_3 Treatment in Hypothyroid Rats

	Euthyroid	*Hypothyroid*	*T_3-6 hours*	*T_3-24 hours*
α-MHC hnRNA	100 ± 3.6%	113 ± 3.5%	115 ± 1.4%	92.3 ± 5.2%
β-MHC AS RNA	100 ± 4.9%	27 ± 0.3%	68 ± 7.6%	52 ± 2.4%

MHC, myosin heavy chain; hnRNA, heterogeneous nuclear RNA; AS, antisense; T_3, triiodothyronine.

measurable levels of β-MHC sense RNA, mRNA, and protein, yielding the well-characterized thyroid hormone–dependent phenotype (1,19).

To confirm that the β-MHC AS RNA molecule is a primary transcript (the complement of the hnRNA), and not a spliced mRNA form of the molecule as has been reported by others, RT PCR was done with the β-MHC mRNA F primer followed by PCR with both β-MHC mRNA primers (35). These primers targeted a region near the 5′ end of the proposed AS mRNA and lie within separate exons. We were not able to amplify β-MHC AS RNA using the β-MHC mRNA primers, confirming that the β-MHC AS RNA is the complement of the hnRNA containing the corresponding intronic and exonic regions.

In the ventricle, the transcription of the β-MHC AS gene appears to be associated with and linked to the transcription of the α-MHC gene; both are induced in the presence of T_3. However, in atria this expression appears to be uncoupled. As observed in the ventricles, the expression of the β-MHC sense and AS genes in the atria is inversely correlated, while the expression of the α-MHC gene is not thyroid hormone responsive and highly expressed in all thyroid states. This observation demonstrates for the first time that the previously identified shared promoter region that lies in the intergenic region between the β-MHC and α-MHC genes is differentially regulated in a tissue-specific manner (10). Exploration of the differences in cofactors and potential epigenetic influences in this shared intergenic promoter region in atria and ventricles may provide additional information regarding the potential mechanism by which T_3 influences the MHC genes in the human heart (5).

Noncoding RNAs identified in recent years include small interfering RNA (siRNA), microRNA (miRNA), and AS RNA. While siRNA (double stranded) and miRNA (single stranded) are small RNAs, usually less than 25 nucleotides or base pairs in length, naturally occurring AS RNAs tend to be longer, poly[A] negative and localized to the nucleus (36). Several thousand AS RNAs have been identified in mammalian genomes, including the human genome, with over 1600 sense–AS pairs transcribed from both DNA strands (37). The MHC genes are differentially regulated in the rat and human myocardium yet demonstrate strikingly conserved regulatory sequences within the α/β intergenic region (10). Human MHC genes are minimally responsive to T_3, in contrast to the rodent myocardium. It is possible that, as we propose in the rat myocardium, the β-MHC gene is constitutively transcribed in the human myocardium and the lack of T_3 responsiveness resides in the α-MHC regulatory region, including the α/β intergenic region. This α/β intergenic region is highly conserved among mammals (10). This would suggest that the mechanistic differences in MHC gene regulation in the rodent and human myocardium are due to either sequence-specific alterations in the intergenic regulatory region that contains potential regulatory sequences for the α-MHC gene as well as the β-MHC AS gene rendering decreased levels of expression of these genes, or that differences in endogenous cofactors that interact with this regulatory region differ in the rodent and human cardiac myocytes. Further studies will help to elucidate the mechanisms by which MHC gene regulation in the human myocardium differs and how it is altered in pathologic cardiac disease states.

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Address reprint requests to:
Irwin Klein, M.D.
North Shore University Hospital
350 Community Drive
Manhasset, NY 11030

E-mail: iklein@nshs.edu